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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/430,175	10/29/1999	STEPHEN A. LESKO	CW-304	6875

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EXAMINER	
CANELLA, KAREN A	
ART UNIT	**PAPER NUMBER**
1642	

DATE MAILED: 12/22/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/430,175	Applicant(s) LESKO ET AL.	
	Examiner Karen A Canella	Art Unit 1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1,2,9-25,28-41,44-56,58-61,64-66 and 68-70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1,2,9-25,28-41,44-48,51-56,58,59,64-66 and 68-70* is/are rejected.

7)☐ Claim(s) *49,50,60 and 61* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *26* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on September 26, 2003 has been entered.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

The request for the correction of inventorship under 37 C.R.F. 1.48(a) (1) is approved. The request will be forwarded to the appropriate official for processing.

Acknowledgment is made of the Declaration under 37 C.R.F. 1.131. However, upon review of the exhibits, dealing with the LnCap cell line, the Declaration is not found to be acceptable in establishing that the instant invention was conceived before October 29, 1998. The instant invention is drawn to the detection of cancer cells in the blood of patients having cancer. It could not have been anticipated or expected that cancer patients would have a level of circulating tumor cells which would be amenable to the instant detection methods comprising density gradient centrifugation followed by labeling with multiple probes based on experiments in which a cancer cell line is labeled with three probes.

Claims 4, 5, 62, 63 and 67 are canceled. Claims 1, 2, 12, 14, 16, 18, 20, 22, 24, 53, 54, 56, 58 and 70 have been amended. Claims 1, 2, 9-25, 28-41, 44-56, 58-61, 64-66 and 68-70 are under consideration.

Claims 49, 50, 60 and 61 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Claim 70 is objected to because of the following informalities: section (b) recites "probes specific cell tumor cell markers" rather than probes specific for tumor cell markers. Appropriate correction is required.

Claims 2, 9, 10, 11, 12, 45, 46 and 51-53are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 2 is vague and indefinite in the recitation of "the single cell" lacks antecedent basis within the claim. The second recitation of "cells" in claim 2 (from a sample containing cells) is vague and indefinite, as it is unclear if said "cells" differ from the first recitation of "cells".

Claim 56 is vague and indefinite because it is unclear which group of "cells" are being referred to.

The recitation of "probe" in claim 11 (versus probes) lacks antecedent basis in claim 1.

The recitation of "cell" (versus cells) in claim 45 lacks antecedent basis in claim 1.

The recitation of "marker" (versus markers) in claim 51 lacks antecedent basis in claim 1.

The recitation of "said epithelial cancer cell" lacks antecedent basis within claim 53 which is drawn to a "epithelial cancer cell preparation" rather than an epithelial cancer cell.

The recitation of "said single cell" in claim 55 lacks antecedent basis in claim 53.

The recitation of "said circulating epithelial cancer cell" in claims 59 and 64-66 lacks antecedent basis in claim 53 which is drawn to an epithelial cancer cell preparation rather than to a circulating epithelial cell.

Claims 1, 2, 9, 10, 11, 12, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70 are rejected under 35 U.S.C. 102(b) as being anticipated by Ts'o (Urology, 1997, Vol. 49, pp. 881-885, reference of the IDS filed September 26, 2003).

Claim 1 is drawn to a method of characterizing single circulating epithelial cancer cells obtained from about 5ml to 75ml blood comprising concurrently measuring multiple cellular markers expressed in said cells using fluorescence microscopy. Claim 2 embodied the method of claim 1 wherein said cells are isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhere onto a surface and fixed with a fixative solution,

and said subsurface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cells and any probe binding with a marker is examined by a microscope equipped with an optical filter set for identification of each specific marker. Claim 9 embodies the method of claim 2 wherein the surface for cell adherence is a microscope slide. Claim 10 embodies the method of claim 2 wherein the fixative is selected from the group consisting of paraformaldehyde, formaldehyde, alcohol or acetone. Claim 56 embodies the method of claim 2 wherein cells are further isolated by a negative selection process. Claim 11 embodies the method of claim 1 wherein said probe is covalently linked to a fluorescent compound that emits a wavelength of light to create a fluorescent probe that binds to a cellular marker. Claim 12 embodies the method of claim 11 wherein said fluorescent probe is selected from other probes with minimal overlapping emission spectra for concurrent use in characterizing said cells. Claim 33 embodies the method of claim 1 wherein the probes comprise multiple fluorescence probes that emit light of different wavelengths with minimal interference between the wavelengths. Claim 34 embodies the method of claim 1 wherein said probe is directed to a cellular target and is not a nucleic acid. Claim 35 embodies the method of claim 34 wherein said probe comprises a protein or peptide. Claim 36 embodies the method of claim 35 wherein said probe in an antibody. Claim 37 embodies the method of claim 1, wherein said probe is a nucleic acid directed to a cellular target. Claims 38 and 38 embody the method of claim 37 wherein said probe comprises DNA and RNA, respectively. Claim 40 embodies the method of claim 1 wherein said probes comprises (I) probes which are directed to a cellular target and are not a nucleic acid, (II) probes which are a nucleic acid directed to a cellular target or (III) a combination of (I) ad (II). Claim 41 embodies the method of claim 40 wherein said probes are selected from the group consisting of identification probes, proliferation probes, cell cycle arrest probes, oncogenes and hormonal probes. Claims 43 and 44 embody the method of claim 40 wherein the probes comprise an epithelial cell-specific probe and a tissue specific probe, respectively. Claim 45 embodies the method of claim 1 wherein said cell is obtained form a mammal. Claim 46 specifies that the mammal of claim 45 is a human. Claim 51 embodies the method of claim 1 wherein said cellular marker is an antigen.

Claim 53 is drawn to a method of characterizing a single epithelial cancer cell preparation obtained form about 5 to 75 ml blood, said method comprising adhering a circulating epithelial

cancer cell preparation to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating said cell surface containing fixed cells with multiple probes directed to desired cellular markers, wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by fluorescence microscopy for identification of positive cells for each selected marker by concurrent measurement of multiple cellular markers, wherein said cancer cell preparation is isolated from a body fluid using a negative selection process, wherein said circulating epithelial cancer cell is obtained. Claim 55 embodies the method of claim 53 wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhered onto a surface and fixed with a fixative solution, and said surface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cell, and any probes binding eighth a marker is examined by a microscope equipped with an optical filter set of identification of each individual marker

Claim 54 is drawn to a method of establishing a characterization profile of a circulating epithelial cancer cell obtained from about 5 ml to 75 ml blood comprising characterizing a single cell environment by concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in the single cell using fluorescence microscopy. Claim 59 embodies any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is a prostatic cancer cell. Claims 64, 65 and 66 embody the method of any one of claims 1, 53 and 54 wherein said circulating epithelial cancer cell is obtained from about 5 to 25 ml of blood, 15-25 ml of venous blood and about 20 ml blood, respectively. Claims 68 and 69 embody the methods of claims 64 and 65, respectively, wherein said epithelial cancer cell is a prostatic cancer cell. Claim 70 embodies the method of any one of claims 1, 53 and 54, wherein said probe are selected from the group consisting of (a)tissue specific probes for determining the cellular origin of the cell, (b) probes specific for tumor cell markers, (c)probes specific for aneuploidy, (d) probes specific for cellular markers of proliferation, (e) probes specific for cellular markers of growth inhibition, (f) probes specific for cell-cycle arrest, (g) probes specific for cellular markers of apoptosis and (h) probes specific for hormonal receptors.

Ts'o disclose a method of characterizing single circulating prostate epithelial cancer cells obtained from about 20 ml blood (page 881, second column, lines 1-2 under the heading "Blood collection") comprising isolating a cellular fraction of the blood (page 883, first column, lines 5-7 under the heading "Prostate Cell detection From Patients with Advanced Prostate Cancer"), and by means comprising isolating by density gradient centrifugation from blood page 882, first column, lines 1-11 under the heading "single Density Gradient Centrifugation"), a sample comprising prostate cancer cells, adhering the sample by contact with an alcohol fixative to a microscope slide page 882, first column, lines 1-4 under the heading "Identification of Isolated Cells"), and incubating the adhered, fixed sample with a multiplicity of probes consisting of anti-PSA monoclonal antibody, polyclonal anti-prostatic acid phosphatase, diaminophenylindole (page 882, second column, lines 1-4) and DNA probes specific for the centromeric regions of chromosomes 7 and 8 (page 882, second column, lines 15-17) wherein each probe reacts with a marker of the prostate cell, and the adhered, fixed sample after contact with the probes is examined by a fluorescent microscope equipped with optical filters for the identification of each specific probe (page 882, second column, lines 4-8). Ts'o doesn't specifically disclose that the probes are covalently linked to a fluorescent compound that emits a wavelength of light to create a fluorescent probe, not does Ts'o et al specifically disclose that the fluorescent probes are chosen to have minimal overlapping emission spectra for concurrent use in characterizing cells. However, this would be an inherent step in the method disclose by Ts'o because the sample of adhered cells is incubated with five fluorescent probes and thus is labeled with five different fluorescent probes. Ts'o et al disclose that the microscope was equipped with filters to allow for the differential detection of various fluorescent dyes (page 882, second column, lines 4-8) as evidenced by Figure 1 which illustrates that green represents immunoreaction with the anti-PS antibody; yellow represents immunoreaction with the anti-PsAP antibody and blue indicates diamidinophenylindole, as well as blue representing chromosome 7 centromere and red representing chromosome 8 centromere. It is apparent from figure 1, legend (E) that human prostate cancer cells were stained green by monoclonal antibody against PSA, blue for chromosome 7 centromere and red for chromosome 8 centromere, thus fulfilling the specific embodiments of multiple probes, concurrent measurement and minimal overlapping emission spectra, thus fulfilling the specific embodiments of claims 1, 2, 9-12, 33, 53-55. The disclosure

of Ts'o further metes the specific embodiments of claims a probe directed to a cellular target that is not a nucleic acid, a probe that is epithelial cell specific and tissue specific (PSA and PsAP)a probe that is an antibody (anti-PSA, anti PsAP), a probe comprising DNA and RNA (probe for centromeres on chromosomes 7 and 8) which contains RNA (tuna as part of the in situ hybridization buffer), probes specific for tumor cell markers, probes for determining the cellular origin of the cells (prostate), probes specific for aneuploidy (Centro mere chromosomes 7 and 8, page 883, first column, last sentence).

Claims 1, 2, 9, 10, 11, 12, 33-41, 43-46, 51, 53-56, 58, 59, 64, 65, 66, 68, 69, 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ts'o (Urology, 1997, Vol. 49, pp. 881-885) in view of Ross (US 5,674,694).

Claim 58 embodies the method of claim 2 wherein cells are further isolated by a positive selection process wherein the specific cell type is selected from a heterogeneous mixture of cells by an antibody that specifically binds to a specific cell type. Ts'o et al teach a negative selection method. Ts'o et al do not teach a negative selection method followed by a positive selection method.

Ross teach that tumor cells may be isolated from hematopoietic cells by either positive selection or negative selection, and if desired, the process used to enrich the tumor cell population may be preformed more than once or in any combination where appropriate (column 8, line 66 to column 9, line 13)..

It would have been be prima facie obvious o one of ordinary skill in the art at the time the invention was made, to further isolate the cancer cells by a positive selection method.

One of ordinary skil in the art would have been motivated to do so with a reasonable expetation of success by the teachings of Ross on the usefulness of both positive and negative selection methods in combination for the isolation of tumor cells.

Claims 1, 2, 9-25, 28, 33-41, 43-46, 51, 53-56, 58, 59, 64-66 and 68-70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ts'o (Urology, 1997, Vol. 49, pp. 881-885) in view of the abstract of Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A108, cited in a previous Office action) and Schlom (In: Molecular Foundation of Oncology, 1991, pp. 95-

134) and Galbraith et al (reference AS15 of the IDS filed January 28, 2001) and Waggoner et al (reference AT15 of the IDS filed January 28, 2001).

Claims 13-25 are drawn to the method of claim 12 wherein the fluorescent compounds have various emission spectra that can be distinguished from each other. Claim 28 is drawn to known fluorescent labels.

Ts'o et al teach the specific embodiments of claims 1, 2, 9, 10, 11, 12, 33-41, 43-46, 51, 53-56, 58, 59, 64, 65, 66 and 68-70 for the reasons set forth above. Ts'o et al do note teach the particular emission spectra of the fluorescent probes used to distinguish the circulating prostatic tumor cells.

The abstract of Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A108, cited in a previous Office action) teaches the improvement in sensitivity and specificity attained by assaying for circulating melanoma cells using multiple markers in place of single markers.

Schlom (In: Molecular Foundation of Oncology, 1991, pp. 95-134) teaches that virtually every property of a tumor cell population has been shown to demonstrate some degree of heterogeneity or modulation and that this includes heterogeneity among cells of the same tumor mass (page 109, second column, lines 1-8 under the heading upregulation of target antigens).

Galbraith et al (reference AS15 of the IDS filed January 28, 2001) teach that fluorescent imaging cytometry is superior to flow cytometry because the method is not limited by the number of fluorophores which may be distinguished (page 592, first column, second full paragraph). Galbraith et al teach that the selection of fluorophores which have spectral properties which are sufficiently separated as to be measured independently, and optimization of the excitation and emission filters in the fluorescent microscope (page 562, first column, third full paragraph).

Waggoner et al (reference AT15 of the IDS filed January 28, 2001) teach multicolor analysis of populations of single cells and a microscope slide surface containing cells (page 499, first column, "Multicolor Immunophenotyping") by quantitative fluorescent imaging (page 499, second column, last paragraph). Waggoner et al teach spectroscopic properties of selected probes (Table 1) which include fluorescent haptens, reagents for determining DNA/RNA

content, and probes for membrane location and fluidity, and physiologic probes sensitive to intracellular calcium and pH.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made, to label the prostate cancer epithelial cells with a multiplicity of probes, wherein said probes were labeled with fluorophores that had spectral properties that were sufficiently separated as to be measured independently of one another. One of ordinary skil in the art would have been motivated to do so with a reasonable expetation of success by the teachings of the abstract of Dale et al on the increased specificity and sensitivity of the detection of circulating melanoma cells by the use of multiple markers which bind to said melanoma cells. One of skill in the art would recognize that by using a greater number of probes for circulating prostate cells, the problem of tumor cell heterogeneity can be minimized. One of skill in the art would recognize that if a give circulating tumor cell has lost expression of a single antigen or antigens it is unlikely that it has lost expression of all antigens, therefore a cocktail of multiple probes will increase the likelihood that any given tumor cell will become labeled.

Claims 1, 2, 9, 10, 11, 12, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over in view of Takeda et al (Cancer, 1996, Vol. 77, pp. 934-940, cited in a prior Office action). .

Claim 47 embodies the method of claim 40 wherein said probes are used to detect a hormone receptor. Claim 48 embodies the method of claim 37 wherein the hormone is an androgen. Claim 52 embodies the method of claim 51 wherein said cellular marker is a receptor.

Ts'o et al teach the specific embodiments of claims 1, 2, 9, 10, 11, 12, 33-41, 43-46, 51, 53-56, 59, 64, 65, 66, 68, 69, 70 for the reasons set forth above. Ts'o et al do not teach the detection of a hormone receptor or an androgen receptor.

Takeda et al teach the immunohistochemical detection of the androgen receptor in patients with prostate carcinoma.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to include a fluorescent probe for the androgen receptor in the imaging cytometry of circulating prostatic epithelial cells as taught by Ts'o et al. one of skill in the art

would have been motivated to do so with a reasonable expectation of success by the teachings of Takeda et al n the prognostic importance of androgen receptor status in prostate cancer patients.

Conclusion

Applicant's submission of an information disclosure statement under 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p) on September 26, 2003 prompted the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 609(B)(2)(i). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308 8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308 3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308 0196.

Karen Canella, Ph.D.
Primary Examiner,
Art Unit 1642
12/15/03